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1. Your reference	GWS\21091	22 FEB 1999
2. Patent application number *(The Patent Office will fill in this part)*	**9904028.9**	23FEB99 E427372-1 D02000 P01/7700 0.00 - 9904028.9
3. Full name, address and postcode of the or of each applicant *(underline all surnames)*	Microbiological Research Authority CAMR Porton Down Salisbury Wiltshire SP4 0JG	
Patents ADP number *(if you know it)*		6566160001
If the applicant is a corporate body, give the country/state of its incorporation	Great Britain	
4. Title of the invention	Neisserial Vaccine	
5. Name of your agent *(if you have one)* "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	MATHYS & SQUIRE 100 Grays Inn Road London WC1X 8AL	
Patents ADP number *(if you know it)*	1081001	

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Claim*(s)*	4
Abstract	-
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11. I/We request the grant of a patent on the basis of this application.

Signature Mathys & Squire

Date 22 February 1999

12. Name and daytime telephone number of person to contact in the United Kingdom

George W Schlich 0171 830 0000

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NEISSERIAL VACCINE

The present invention relates to vaccines and methods for preparing vaccines that confer protective immunity to infection by *Neisseria*. In particular, the present invention relates to vaccines based on commensal *Neisseria* such as *N lactamica* or components or extracts thereof that provide broad spectrum protective immunity to infection.

Meningococcal meningitis is a major problem worldwide and in many countries the incidence of infection is increasing. *Neisseria meningitidis* is the causative agent of the disease and is also responsible for meningococcal septicaemia, which is associated with rapid onset and high mortality, with around 22% of cases proving fatal.

At present, vaccines directed at providing protective immunity against meningococcal disease provide only limited protection because of the many different strains of *N. meningitidis*. Vaccines based upon the serogroup antigens, the capsular polysaccharides, offer only short lived protection against infection and do not protect against many strains commonly found in North America and Europe. A further drawback of these vaccines is that they provide low levels of protection for children under the age of 2 years, one of the most vulnerable groups that are commonly susceptible to infection.

Gold et al (the Journal of Infectious Diseases, volume 137, no. 2, February 1978, pages 112-121) have reported that carriage of *N. lactamica* may assist in the development of natural immunity to *N. meningitidis* by induction of cross-reactive antibodies. This conclusion was based on the observation of cross-reacting antibodies having complement-dependent bactericidal activity produced in response to *N. lactamica* infection. However, Cann and Rogers (J. Med. Microbial., volume 30, 1989, pages 23-30) detected antibodies to common antigens of pathogenic and commensal neisseria

species, but observed also that antibody to the same antigens was present in both bactericidal and non-bactericidal sera. Thus, it did not seem possible to identify the basis for any cross-reactive bactericidal antibodies, if such exist.

Live attenuated vaccines for meningococcal disease have been suggested by Tang et al (Vaccine 17, 1999, pages 114-117) in which a live, attenuated strain of *N. meningitidis* could be delivered mucosally. Tang also commented on the use of commensal bacteria to protect against infection by pathogenic bacteria, concluding that the cross-reactive epitopes that induce protection against meningococcal infection have not been defined, and therefore that use of genetically modified strains of *N. meningitidis* would be preferred.

It is desirable to provide a further vaccine that gives protective immunity to infection from *N. meningitidis.* It further is desirable to provide a vaccine that confers protective immunity to infants as well as adults and whose protection is long term. It would be of advantage to provide a vaccine that protects against sub-clinical infection, i.e. where symptoms of meningococcal infection are not immediately apparent and the infected individual may act as a carrier of the pathogen. It would further be of advantage to protect against all or a wide range of strains of *N. meningitidis*. It is still further desirable to provide a vaccine against other Neisserial infection, notably gonorrhoea.

It is an object of the present invention to provide compositions containing immunostimulating components, and vaccines based thereon, that meet or at least ameliorate the disadvantages in the art.

The present invention is based on the use of *N. lactamica*, a commensal *Neisseria*, as a vaccine against meningococcal disease, and *N. lactamica* may be used as a live vaccine or a killed whole cell vaccine, or in a vaccine containing fractions of *N. lactamica*. It has surprisingly been demonstrated

that mice immunised according to the present invention with *N. lactamica* killed whole cells and outer membrane preparations are protected from lethal intraperitoneal meningococcal challenge, and that vaccines composed of a detergent extract of *N. lactamica* cells or fractions of this, separated by preparative electrophoresis, also protect mice from lethal meningococcal challenge. These results have been obtained using mice and the mouse model used is regarded as predictive of corresponding immunogenic and vaccinating effects in humans.

Accordingly, a first aspect of the present invention provides a composition for vaccination against Neisserial infection, comprising a commensal *Neisseria* or an immunogenic component or extract thereof and a pharmaceutically acceptable carrier.

It is an advantage of the invention that vaccination against Neisserial diseases may thus be achieved using a non-pathogenic species of *Neisseria*, rendering the vaccination a safer procedure. Furthermore, the protection conferred surprisingly may not restricted to a specific serotype, subtype or serogroup but is of general protective efficacy.

A number of different commensal *Neisseria* are suitable for use in the invention, and these commensal *Neisseria* may be selected from the group consisting of *N. lactamica, N. cinerea, N. elongata, N. flavescens, N. polysaccharea, N. sicca* and *N. subflava*. Different species of these commensal organisms are known to colonise the buccal or nasal areas and hence each species may be administered according to the known area of the body it normally colonises. Hence also, use of a composition of the invention may result in stimulation of production of protective antibodies *de novo* or if the patient has already been colonised to a certain extent may result in an enhancement of naturally-existing antibodies.

A still further advantage of the invention is that the commensal *Neisseria*

that are the subject of the invention can not revert to virulent types, which can be a risk in the use of live, attenuated strains of virulent bacteria. It is known in the vaccination field to use live, attenuated pathogens and this use carries the risk that the attenuated organism may revert to virulence. This risk is avoided by the present invention. Furthermore, *N. meningitidis* possesses many virulence factors the precise roles of which in pathogenesis are unknown and may posses hitherto unrecognised virulence factors. Therefore, an additional advantage of the invention is that a composition of the invention can be used with confidence in its level of safety.

The method of the invention is of application to vaccination against various Neisserial infections. In a specific embodiment of the invention, protection against meningococcal disease, more specifically caused by *N. meningitidis*, has been demonstrated. The invention is also of application to vaccination generally against Neisserial infection, including gonorrhoeal infection.

The composition can specifically comprise killed *N.lactamica*, which may for example be obtained by heat or by suspending *N.lactamica* in a mixture of bactericidal agents such as thiomersal and formaldehyde.

The composition may also comprise live *N. lactamica*. As mentioned it is usually not required to use attenuated *N. lactamica* as this organism is avirulent.

In an embodiment of the invention, an immunogenic component or extract of *N.lactamica* is selected from an outer membrane preparation of *N.lactamica*, *N.lactamica* lipooligosaccharide and a protein fraction of *N.lactamica*.

The outer membrane preparation and protein fraction of *N.lactamica* can be obtained from *N.lactamica* cultured in the presence or absence of iron. The protein fraction of *N.lactamica* is conveniently obtained by suspending

N.lactamica cells or membranes in the presence of detergent and incubating the suspension so as to extract proteins from the *N.lactamica*.

Alternatively, a number of other techniques are known for extraction of protein fractions from cell preparations and are suitable to obtain the commensal *Neisseria* protein fraction of the invention. Examples of conventional techniques for this purpose include the use of variation in salt concentration, chaotropic agents, variation in pH (high or low), enzymic digestion and mechanical disruption.

A number of different fractions are suitable for use in vaccinating against meningococcal disease. Particularly suitable fractions are those of molecular weight less than 40kDa, of molecular weight more than 40kDa and less than 67kDa, and of molecular weight more than 67kDa.

In more specific embodiments of the invention there is provided a composition for eliciting an immune response and suitable for use in vaccinating an individual against Neisserial infection, more specifically meningococcal disease, comprising an antigenic component or antigenic components having the properties:-

(a) molecular weight 50kDa or lower;

(b) obtainable from *N. lactamica;* and

(c) antibodies to the component(s) obtained from *N. lactamica* cross-react with *N. meningitidis.*

In use of a composition containing such a component, extracted using detergent, all mice treated with this component survived a challenged dose of 2×10^7 CFU *N. meningitidis* and three out of five mice survived a higher challenge dose of 6×10^8 CFU.

Another specific embodiment of the invention lies in a composition for eliciting an immune response and suitable for use in vaccinating an individual

against Neisserial infection, more specifically meningococcal disease, comprising an antigenic component or antigenic components having the properties:-

(a) molecular weight at least 40kDa and up to 90kDa;

(b) obtainable from *N. lactamica;* and

(c) antibodies to the component(s) obtained from *N. lactamica* cross-react with *N. meningitidis.*

In use of such a component of the invention, obtained using a detergent extract of *N. lactamica*, four out of five mice treated with the component survived a challenge dose of 2×10^7 CFU *N. meningitidis* and mice receiving a higher challenge dose of 6×10^8 CFU survived longer than a control group.

A still further embodiment of the invention lies in a composition for eliciting an immune response and suitable for use in vaccinating an individual against Neisserial infection, more specifically meningococcal disease, comprising an antigenic component or antigenic components having the properties:-

(a) molecular weight at least 70kDa;

(b) obtainable from *N. lactamica;* and

(c) antibodies to the component(s) obtained from *N. lactamica* cross-react with *N. meningitidis.*

In use of such a component, obtained using a detergent extract, one out of five mice survived a challenge dose of 2×10^7 CFU *N. meningitidis* and, whilst all mice succumbed to a higher challenged dose of 6×10^8 CFU, their survival time was longer than a control group which did not receive the component.

By way of example of a method of extracting an antigenic component of the invention, an extraction method comprises:-

(i) suspending *N.lactamica* cells in an aqueous solution of

detergent;

(ii) incubating the suspension so as to extract the antigenic component from the *N.lactamica*;

(iii) centrifuging the suspension to separate the suspension into a supernatant and a pellet; and

(iv) fractionating the antigenic component from the supernatant.

This specific method can be modified according to the extraction protocol selected by the user, for example by using high salt concentration in the initial step (i). In further embodiments of the invention the antigenic component is obtained using recombinant technology by expression of a *N. lactamica* sequence in a suitable host such as *E. coli*.

In a second aspect of the invention there is provided a composition for vaccination against Neisserial infection comprising a commensal *Neisseria* and a pharmaceutically acceptable carrier, wherein the commensal *Neisseria* comprises and expresses a gene from a pathogenic *Neisseria*.

This aspect of the invention offers the benefit of use of a commensal organism to deliver and/or present to the recipient an antigen from a pathogenic *Neisseria*. The gene optionally encodes a surface antigen or a protein that is secreted, and may code for an antigen from, for example, *N. meningitidis* or *N. gonorrhoea*. The commensal *Neisseria* can be live or killed.

In an embodiment of the second aspect of the invention there is provided a composition for vaccination against meningococcal disease comprising a commensal *Neisseria* and a pharmaceutically acceptable carrier, wherein the commensal *Neisseria* comprises and expresses a *N. meningitidis* gene.

The *N. meningitidis* gene may encode a transferrin binding protein, a SOD for example a Cu,Zn-superoxide dismutase ("SOD"), Neisserial surface protein A ("NspA"), a porin or another outer membrane protein. Gene sequences for the majority of these antigens are known in the literature. Kroll et al in Microbiology 141 (Pt 9), 2271-2279 (1995) describe the sequence of Cu,Zn-SOD. Martin et al in J Exp Med, 1997, April 7th, 185(7), pp1173-1183 describe the sequence of NspA from *N. meningitidis*.

The invention also provides a pharmaceutical composition comprising a composition according to the first or second aspect of the invention plus a pharmaceutically acceptable carrier.

In a third aspect, the invention provides a method of vaccination against Neisserial infection, comprising administering an effective amount of a composition according to the first and second aspects of the invention.

In use of an embodiment of the invention described in an example below, there is provided a method of vaccination against meningococcal disease, comprising administering an effective amount of a composition according to the first and second aspects of the invention.

In a fourth aspect of the invention there is provided a strain of a commensal *Neisseria*, such as *N. lactamica*, genetically modified so as to express a gene from a pathogenic *Neisseria*. The *N.meningitidis* gene may code for a protein selected from a transferrin binding protein, a SOD for example a Cu,Zn-SOD, NspA, a porin or another outer membrane protein.

The invention further provides, in a fifth aspect a method of extracting a protein for incorporation in a composition suitable for vaccinating against meningococcal disease, comprising:-

(i) suspending *N.lactamica* cells in the presence of detergent; and

(ii) incubating the suspension so as to extract a protein fraction from the cells.

The protein fraction can suitably be of molecular weight 50kDa or lower, at least 40kDa and up to 90kDa or at least 80kDa.

The composition may be combined with a pharmaceutically acceptable carrier - for example the adjuvant alum although any carrier suitable for oral, intravenous, subcutaneous, intraperitoneal intramuscular, intradermal or any other route of administration is suitable - to produce a pharmaceutical composition for treatment of meningococcal disease. Commensal *Neisseria* that are buccal colonizers can be administered in a mouthwash and nasal colonizers in a nasal spray.

Transferrin binding proteins are known to be located on the outer membranes of a number of Gram negative bacteria such as *N. meningitidis*. Formulations of the composition of the present invention with conventional carriers or adjuvants and optionally further supplemented by one or more antigens from *Neisseria* species, optionally recombinantly produced, for example, Cu-Zn SOD, the 22kD NspA, porins, gonorrhoeal antigens or transferrin binding proteins provide a composition for treatment of infection by these bacteria.

In the present invention, the term "transferrin binding protein" or "Tbp" refers to a protein which either alone binds to transferrin or can be part of a complex of proteins that binds transferrin. The term also embraces fragments, variants and derivatives of such a protein provided that antibodies raised against the fragment, variant or derivative bind the protein. Thus, TbpA and TbpB either dissociated or associated into a complex are considered to be Tbp. Moreover, mutants, fusion proteins or fragments of either TbpA or B or other derivatives of the TbpA+B complex with a common antigenic identity are also considered to be represented by the term

Tbp in the present invention.

A live vaccine according to the present invention may be administered via intranasal inoculation. A killed bacteria or subunit vaccine may also be given by this route, or formulated for oral delivery. It is most likely that a subunit vaccine would be administered via the parenteral route. Different commensal *Neisseria* and different strains of *N. lactamica* from those tested in specific embodiments of the invention exist, and the invention is of application also to those other strains.

A still further aspect of the invention provides a composition comprising an antibody, wherein the antibody binds to a commensal *Neisseria* or an immunogenic component or extract thereof. In use, the antibody can be formulated into a pharmaceutical composition for treatment of Neisserial infection, such as meningococcal disease or infection caused by other *Neisseria*.

An antibody according to this aspect of the invention can be obtained following standard techniques by inoculating an animal with a commensal *Neisseria* or an immunogenic component or extract thereof and thereafter isolating antibodies that bind to the commensal *Neisseria* or the immunogenic component or extract thereof.

Specific embodiments of the invention are discussed in more detail by means of the Examples described below. The results referred to in the Examples are illustrated by the accompanying drawings, in which:

Fig.1 shows protection of mice against intraperitoneal ("IP") infection with *N.meningitidis* strain K454 by use of *N.lactamica* whole cells and outer membrane fractions;

Fig.2A shows protection of mice against IP infection with *N.meningitidis* strain K454 by use of detergent and high, medium and low molecular weight extracts of *N.lactamica* cells - upper panel = challenge by

2×10^7 CFU, lower panel = challenge by 6×10^8 CFU;

Fig.2B shows the components of the high, medium and low molecular weight fractions of fig.2A; and

Fig.3 shows an immunoblot illustrating cross-reaction of antibodies in sera from meningococcal disease patients with proteins from *N.lactamica* strain Y92-1009.

Example 1

Preparation of vaccine containing killed whole cells

Neisseria lactamica strain Y92-1009 was grown in Mueller Hinton broth (MHB) containing 5μgml^{-1} ethylenediamine-di(o-hydroxyphenylacetic acid) (EDDHA), incubated at 37°C with shaking (140rpm) for approximately 6h.

Bacteria were then harvested by centrifugation and resuspended in phosphate buffered saline (PBS) containing 1% (v/v) formaldehyde and 0.1% (w/v) thiomersal, and left to stand overnight at 2-8°C. Killed cells were then resuspended in PBS to an OD_{650} of 1.0 (equivalent to 2×10^9 CFUml^{-1}) and alhydrogel added to 25% (V/V), yielding a product suitable for subcutaneous administration.

This method is suitable also for *N. cinerea, N. elongata, N. flavescens, N. polysaccharea, N. sicca* and *N. subflava*.

Example 2

Preparation of vaccine containing *N. lactamica* outer membrane (OM) preparations

N. lactamica strain Y92-1009 was grown in MHB with and without the addition of 5μgml^{-1} EDDHA overnight at 37°C with shaking. Iron limited

(with EDDHA) and iron replete cells were then treated separately. Bacteria from 1.5 litres were harvested by centrifugation and resuspended in 20ml 200mM Lithium acetate, 5mM EDTA, pH 6.0 and incubated for 3h at 37°C with shaking. Bacteria were then passed 7 times through a 21 gauge needle and pelleted at 8000g for 10min.

The supernatant was recovered and membranes pelleted by centrifugation at 100,000g for 1h at 4°C. The membranes were then resuspended in 10mM HEPES, pH 7.4, containing 0.1% (v/v) 10mM PMSF, yielding OM-containing vaccinating preparations. The protein content of the OM vaccine preparations was determined using the bicinchoninic acid assay (Sigma, UK). OMs were diluted in sterile deionized water to give a protein concentration of 100μgml^{-1}. This was then mixed with an equal volume of Freund's adjuvant, to give a final protein concentration of 50μgml^{-1}, and emulsified thoroughly. Freund's complete adjuvant was used for the primary dose, and Freund's incomplete for subsequent boosts.

Example 3

Preparation of vaccine containing lipooligosaccharide (LOS)

Purification of LOS was carried out from *N. lactamica* strain Y92-1009 using the method of Gu, X-X and Tsai, C.M. (1991) Anal Biochem. **196**; 311-318. Vaccine was prepared using Freund's adjuvant as above with LOS at a final concentration of 10μgml^{-1}.

Example 4

Vaccination and challenge schedule

Groups of 5 mice were vaccinated with each preparation as follows:-

Prime:- Day 0

First boost:- Day 21
Second boost:- Day 28

Mice vaccinated with killed cells of Example 1 received 0.5ml subcutaneously, equivalent to 1 x 10^9 CFU. Mice vaccinated with OM of Example 2 and LOS of Example 3 received 0.2ml subcutaneously; equivalent to 10μg of protein and 2μg of LOS.

On day 35, mice were challenged by intraperitoneal injection with approximately 10^8 CFU *N. meningitidis* K454 made up in MHB containing transferrin at a final concentration of 20mg/ml. The mice were then examined and the number of survivors noted and the results are shown in fig.1. After 4 days all 5 mice survived in the groups vaccinated with whole cells and OMPs (with iron) and 3 survived in the group vaccinated with OMPs (without iron). After 5 days all members of the control group and of the group vaccinated with LOS (marked LPS on the figure) had died.

Example 5

Preparation of vaccine comprising *N. lactamica* fractions

Brain heart infusion agar plates were inoculated with 50μl of *N. lactamica* strain Y92-1009 and incubated overnight at 37°C, with 5% CO_2. This was used to inoculate a 100ml MHB starter culture which was incubated with shaking at 37°C for 6 h. Starter culture (15ml) was added to each of 6x500ml volumes of MHB. These were then incubated with shaking overnight at 37°C and the conditions were made iron-limited by the addition of 5ugml^{-1} EDDHA. The cells were harvested by centrifugation and the supernatant discarded. The cells were washed with 100ml PBS and then pelleted by centrifugation. Cell pellets were resuspended in PBS + 0.3% (v/v) Elugent (Calbiochem, 2ml per g wet weight) and incubated with shaking at 37°C for 20 min. The cells were then removed by centrifugation

and the pellet discarded. EDTA and N-lauroyl sarcosine were then added to the supernatant to 10mM and 0.5% (w/v) respectively.

The BIORAD Prep Cell, model 491 was then used to separate the proteins contained in the detergent extract. A 4cm, 7% acrylamide native resolving gel was cast with a 2 cm stacking gel. 12mg of protein in native sample buffer was electrophoresed using running buffer containing 0.1% (w/v) SDS, 0.025M Tris and 0.192M glycine at 40mA and 400V until the dye front reached the bottom of the gel. 3ml fractions of the eluted proteins were then collected. Once the fractions were collected they were pooled into groups consisting of proteins of molecular weight approximately less than 40kDa, between 40 and 67kDa and more than 67kDa. The pooled proteins were concentrated by ammonium sulphate precipitation and dialysed against PBS. These were diluted in PBS to a protein concentration of 100ug/ml and Freund's complete adjuvant was added at a ratio of 1:1(v/v) or Freund's incomplete adjuvant for booster doses.

Example 6

Vaccination and challenge schedule

Groups of 5 mice were vaccinated with each preparation as follows:-

Prime:-	Day 0
First boost:-	Day 21
Second boost:-	Day 28

Mice were vaccinated with no vaccine (i.e control group), Elugent ("Registered Trade Mark") extract or high, medium or low molecular weight fraction. The mice receiving the protein fraction groups received 0.2ml subcutaneously; equivalent to 10μg of protein.

On day 35 mice were challenged by intraperitoneal injection with either

approximately 2×10^7 or 6×10^8 CFU *N. meningitidis* K454 made up in MHB containing transferrin at a final concentration of 20mg/ml. The mice were then examined over four days and the number of survivors noted, and the results are shown in fig.2A - upper panel 2×10^7 challenge and lower panel 6×10^8 challenge. The components of the high, medium and low molecular weight fractions are shown in fig.2B. after being run an SDS-PAGE gel.

Example 7

We investigated samples of human sera following meningococcal disease and these showed that antibodies were produced which react with a range of *N. lactamica* proteins. The results of the immunoblot are shown in fig.3.

The invention thus provides immunogenic compositions and vaccines for use in protecting against meningococcal disease.

CLAIMS

1. A composition for vaccination against Neisserial infection, comprising a commensal *Neisseria* or an immunogenic component or extract thereof and a pharmaceutically acceptable carrier.

2. A composition according to Claim 1 comprising killed *Neisseria*.

3. A composition according to Claim 2 wherein killed *N.lactamica* are obtained by suspending *N.lactamica* in a mixture of bactericidal agents such as thiomersal and formaldehyde.

4. A composition according to Claim 1 comprising live *N. lactamica*.

5. A composition according to Claim 1 wherein the immunogenic component or extract of *Neisseria* is selected from an outer membrane preparation, a lipooligosaccharide and a protein fraction.

6. A composition according to Claim 5 wherein the *Neisseria* is *N. lactamica*.

7. A composition according to Claim 5 wherein the immunogenic component or extract comprises a protein fraction of molecular weight less than 40kDa.

8. A composition according to Claim 5 wherein the immunogenic component or extract comprises a protein fraction of molecular weight more than 40kDa and less than 67kDa.

9. A composition according to Claim 5 wherein the immunogenic component or extract comprises a protein fraction of molecular weight more than 67kDa.

10. A composition according to any of Claims 7 to 9 wherein a protein fraction of *N.lactamica* is obtained by suspending *N.lactamica* cells in the presence of detergent and incubating the suspension so as to extract proteins from the *N.lactamica*.

11. A composition for eliciting an immune response and suitable for use in vaccinating an individual against Neisserial infection, comprising an antigenic component having the properties:-
(a) it is of molecular weight 50kDa or lower;
(b) it is obtainable from a commensal *Neisseria*; and
(c) antibodies to the component obtained from the commensal *Neisseria* cross-react with *N. meningitidis.*

12. A composition for eliciting an immune response and suitable for use in vaccinating an individual against Neisserial infection, comprising an antigenic component having the properties:-
(a) it is of molecular weight at least 40kDa and up to 90kDa;
(b) it is obtainable from a commensal *Neisseria*; and
(c) antibodies to the component obtained from the commensal *Neisseria* cross-react with *N. meningitidis.*

13. A composition for eliciting an immune response and suitable for use in vaccinating an individual against Neisserial infection, comprising an antigenic component having the properties:-
(a) it is of molecular weight at least 70kDa;
(b) it is obtainable from a commensal *Neisseria*; and
(c) antibodies to the component obtained from the commensal *Neisseria* cross-react with *N. meningitidis.*

14. A composition according to any of Claims 11 to 13 further comprising one or more of a transferrin binding protein, a Cu,Zn-SOD, a porin and NspA.

15. A composition for vaccination against Neisserial infection comprising a commensal *Neisseria* and a pharmaceutically acceptable carrier, wherein the commensal *Neisseria* expresses a gene from a pathogenic *Neisseria*.

16. A composition according to Claim 15 wherein the commensal *Neisseria* expresses a gene which encodes a transferrin binding protein from *N. meningitidis*.

17. A composition according to Claims 15 or 16 wherein the commensal *Neisseria* expresses a gene which encodes a Cu,Zn-SOD from *N. meningitidis*.

18. A composition according to any of Claims 15 to 17 wherein the commensal *Neisseria* expresses a gene which encodes a NspA from *N. meningitidis*.

19. A composition according to any of Claims 15 to 18 wherein the commensal *Neisseria* expresses a gene which encodes a porin from *N. meningitidis*.

20. A pharmaceutical composition comprising a composition according to any of Claims 1 to 19 plus a pharmaceutically acceptable carrier.

21. A method of vaccination against Neisserial infection, comprising administering an effective amount of a composition according to any of Claims 1 to 20.

22. A commensal *Neisseria* strain, which expresses a gene from a pathogenic *Neisseria*.

23. A strain of *N.lactamica* according to Claim 22, which expresses a

N.meningitidis gene which codes for a protein selected from a transferrin binding protein, a porin, NspA, an outer membrane protein, and a Cu,Zn-SOD.

24. A method of extracting a protein for incorporation in a composition suitable for vaccinating against meningococcal disease, comprising:-

    (i) suspending *N.lactamica* cells in the presence of detergent; and

    (ii) incubating the suspension so as to extract a protein fraction from the cells.

25. A method according to Claim 24, wherein the protein fraction is of molecular weight 50kDa or lower.

26. A method according to Claim 24, wherein the protein fraction is of molecular weight at least 40kDa and up to 90kDa.

27. A method according to Claim 24, wherein the protein fraction is of molecular weight at least 80kDa.

28. A method according to any of Claims 24 to 27 further comprising mixing the protein fraction with one or more of a transferrin binding protein, a Cu,Zn-SOD, a porin and NspA.

29. A pharmaceutical composition comprising an antibody that binds to a commensal *Neisseria* or an immunogenic component or extract thereof and a pharmaceutically acceptable carrier.

30. A composition for vaccinating against meningococcal disease substantially as hereinbefore described.

Fig. 1. Protection of mice against IP infection with *N.meningitidis* strain K454 by vaccination with *N. lactamica* whole cells and outer membrane proteins

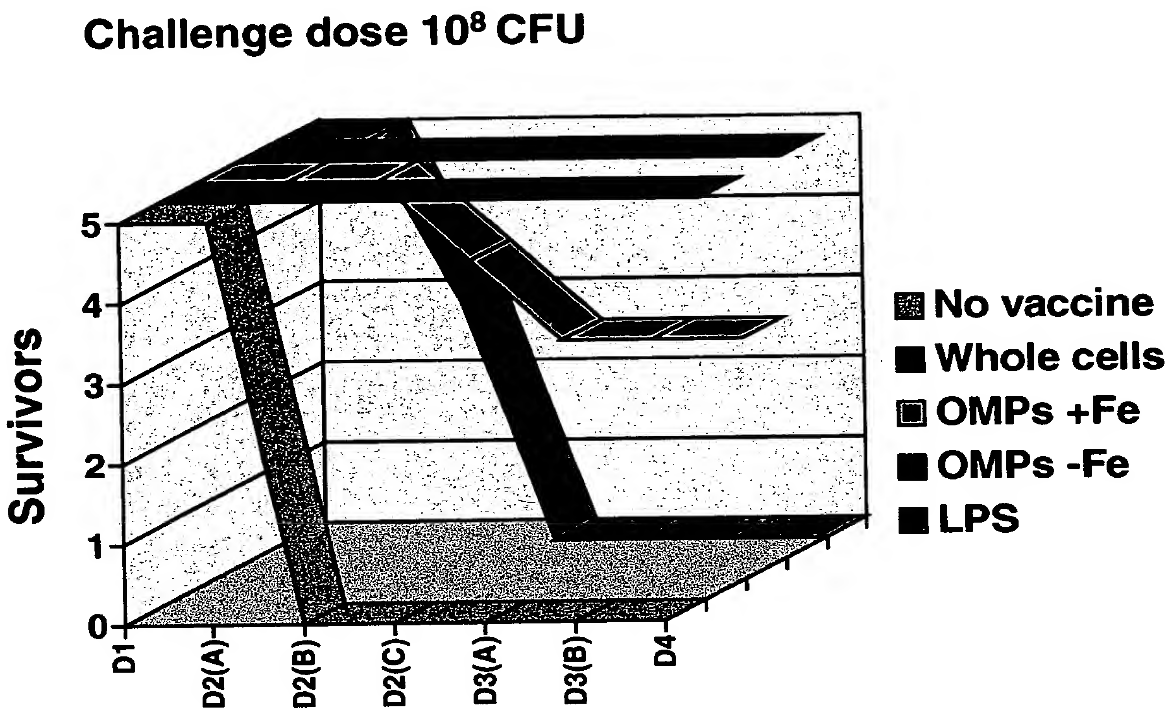
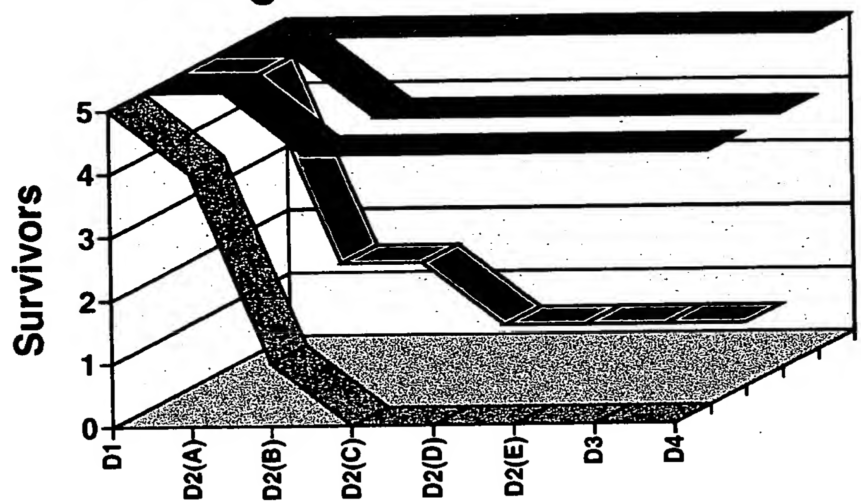


Fig. 2A. Protection of mice against IP infection with *N. meningitidis* strain K454 by vaccination *N. lactamica* extracts

Challenge dose 2 x 10^7 CFU



No vaccine ■ Elugent extract ▣ High ■ Medium ■ Low molecular weight fractions

Challenge dose 6 x 10^8 CFU

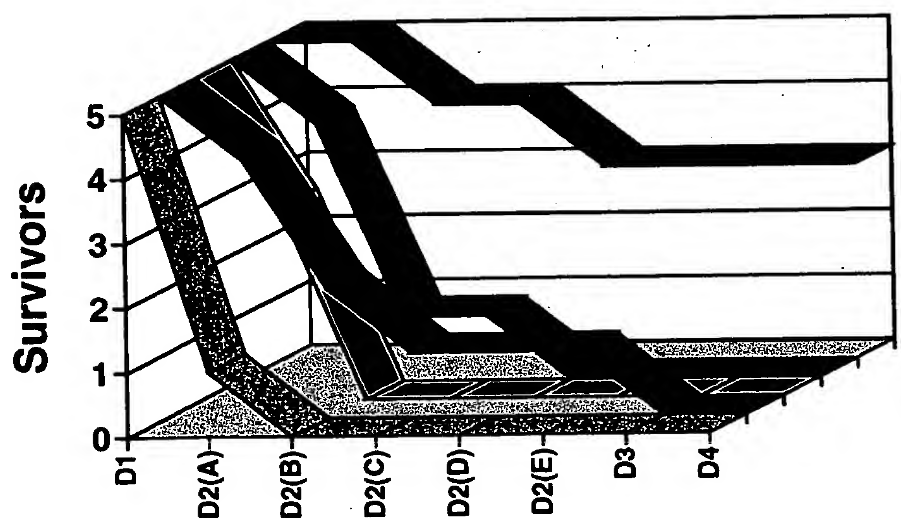


Fig. 2B. SDS-PAGE of proteins separated by preparative electrophoresis used in protection expt.

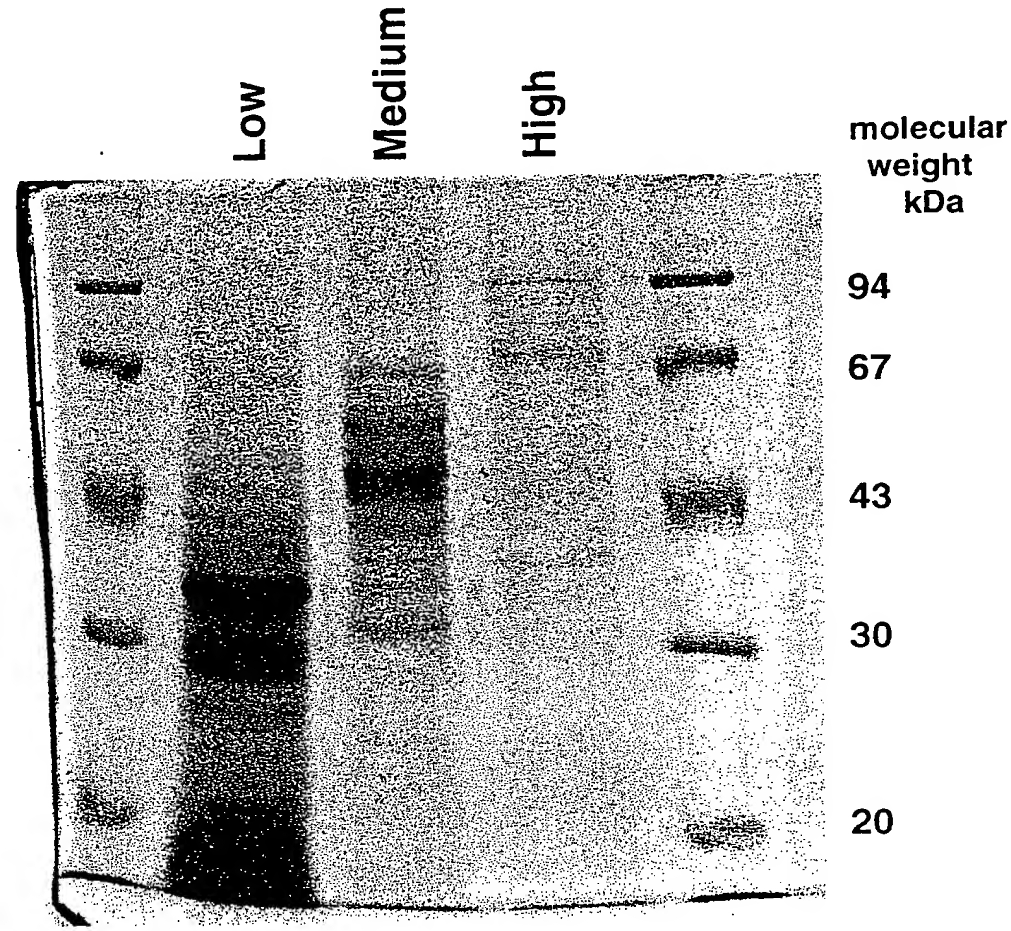


Fig 3. Immunoblot showing cross-reaction of antibodies in sera from meningococcal disease patients with proteins from *N. lactamica* strain Y92-1009